

9 Jan 2000

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CHANGES IN BLOOD VOLUME***

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20010424 025

***Report No. 00-40***

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Report Number 00-40, supported by the Office of Naval Research, Arlington, VA, under Work Unit No. 64771N.M2443.001.6901. The views expressed in this paper are those of the authors and do not reflect official policy or position of the Department of the Navy, the Department of Defense, or the U.S. Government. Approved for public release; distribution unlimited.

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## Summary

### Problem

Combat casualty care requires that battlefield traumas be quickly and accurately assessed, and that patients' vital signs be continuously monitored to immediately detect small fluctuations that may have major implications for needed life-saving measures. Biomedical technologies are needed that will allow rapid initial assessment of the patient's condition and continuous monitoring of critical autonomic nervous system (ANS) activity.

### Objective

The purpose of this study was to evaluate the effect of simulated changes in blood volume on ANS activity and balance using spectral analysis of heart rate variability (HRV).

### Approach

Eight male subjects completed 3 randomized 4-hr trials of seated rest (CON), 6° head-down tilt (HDT), and seated water immersion (WI) to midchest. Before and after each trial, body weight was measured, urine collected, and blood samples drawn for analysis of pre- and post-trial hematocrit values (Hct) and calculation of percent changes in plasma volume. Heart rate (HR) was measured continuously throughout each trial. From HR, real-time spectral analysis was conducted on successive 32-sec time segments (512 values) for the determination of high-frequency (Rfa) and low-frequency (Lfa) amplitudes. Additional measures included the Lfa-to-Rfa ratio (LRR), fundamental respiratory frequency (FRF), and 32-sec mean HR (mHR).

### Results

Measures of body weight loss, urine output, and change in plasma volume over time were consistent with successful simulations of blood loss among the HDT and WI groups. Analysis of variance revealed significant trial effects for Lfa and Rfa, and significant trial, time, and trial-by-time interaction for mHR. There were no significant differences for LRR or FRF among trials.

### Conclusion

The present study provided no additional support to the previously proposed notion that LRR may be a useful indicator of ANS balance.

## **Heart Rate Variability and Changes in Blood Volume**

### **Introduction**

Combat casualty care requires that battlefield traumas be quickly and accurately assessed, and that patients' vital signs be continuously monitored to immediately detect small fluctuations that may have major implications for needed life-saving measures. Consequently, biomedical technologies are needed that will allow rapid initial assessment of the patient's condition and continuous monitoring of critical autonomic nervous system (ANS) activity.

The ANS controls and coordinates the function of all visceral organs to promote homeostasis.<sup>1</sup> These include heart and lung function, blood flow, blood pressure regulation, thermoregulation, and reactions to stress and fatigue. With respect to the heart, the autonomic nervous system controls heart rate (HR) through the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). The PNS regulates HR by sending efferent impulses via the vagus nerve to the sinoatrial node in the heart, while the SNS has its regulatory impact on HR by sending efferent sympathetic impulses to the ventricles. Interaction of the PNS and SNS produces beat-to-beat heart rate variability (HRV).<sup>2</sup>

Heart rate variability is reflective of ANS activity and the balance between the PNS and SNS,<sup>3</sup> and may be examined using time domain or frequency domain analysis. Time domain analysis consists of differing standard deviation calculations of HR, or heart period, defined as beat-to-beat intervals over time. Frequency domain analysis consists of either autoregressive or Fast Fourier transformation techniques to delineate frequency components over a range of 0.0 Hz to 0.5 Hz. The power component in the high-frequency (HF) oscillations range from 0.15 Hz to 0.5 Hz and is correlated with respiratory-mediated baroreflex modulation of cardiac vagal tone. The component in the low-frequency (LF) band from 0.05 to 0.15 Hz is thought to be a quantitative marker of sympathetic activity. However, some researchers view LF as an indicator of both sympathetic and vagal activity.<sup>3</sup> The power in the very-low-frequency (VLF) band (<0.05 Hz) has been linked with the renin-angiotensin system, temperature regulation, and slow vasomotor activity.

Acute blood loss provokes a multitude of compensatory and decompensatory circulatory and metabolic responses.<sup>4,5</sup> The decompensatory reactions can lead directly to cardiac failure,

acidosis, central nervous system depression, aberrations of blood coagulation, and depression of the reticuloendothelial system. Further, the compensatory responses activate baroreceptor and chemoreceptor reflexes, limit cerebral ischemia, redistribute tissue fluids, release endogenous vasoconstrictors, and promote renal conservation of water and electrolytes. These responses can lead to hypovolemia, hypotension, bradycardia, renal water and electrolyte excretion, increases in atrial natriuretic peptide, and changes in plasma vasopressin, plasma renin activity, and aldosterone.

Simulation of the negative-feedback compensatory cardiovascular changes associated with hemorrhaging may be accomplished through water immersion (WI) and head-down tilt (HDT). During WI, venous blood is shifted from the legs to the torso because of an increase in external pressure. However, during HDT redistribution of blood occurs as a result of a reduction in hydrostatic pressure. In both cases, the accumulation of blood in the thoracic region increases central blood volume and intracardiac pressure. These changes promote an increase in urinary fluid and electrolyte loss and a decrease in extracellular volume and plasma volume.<sup>6</sup> However, while WI and HDT produce similar outcomes, the pattern and magnitude of the responses are different. Compared with HDT, WI produces an initial hemodilution and then greater increases in urine volume and a concomitant loss of plasma proteins.<sup>7</sup>

Miwa et al.<sup>8</sup> reported that WI decreases heart rate and increases stroke volume and cardiac output. These changes were also accompanied by increased HF fluctuations and reduced LF fluctuations in HR. However, Perini et al.<sup>9</sup> reported that WI is associated with large increases in the VLF, LF, and HF components. The major limitation of these studies were the short durations of WI, lasting only 5 to 15 min, and the absence of demonstrated changes in body fluid volumes. The impact of longer durations of WI and HDT on HRV, especially after significant reductions in body fluid volumes, has not been studied. Therefore, the present study seeks to evaluate changes in HRV over a time period likely to produce a modest reduction of extracellular fluid volume.

## **Methods**

### **Subjects.**

Subjects were eight active-duty Navy or Marine Corps personnel (age  $22.1 \pm 5.4$  years; height  $172.0 \pm 5.0$  cm; weight  $75.6 \pm 13.4$  kg). All subjects were briefed on the study protocol and gave informed consent before participating. All subjects completed medical history questionnaires.

Medical records were reviewed by a physician to ensure that subjects were medically qualified to participate in the study.

#### Experimental Procedures.

Eight male subjects completed three randomized trials of 4-hrs (240 min) duration of seated rest (CON), 6° head-down tilt (HDT), and seated water-immersion (WI) to midchest in 35°C water. Subjects wore shorts during all trials, and watched nonexcitatory videos (movies from the 1950s and 1960s) on a television monitor to pass the time. The previous night and the morning of each test, subjects were instructed to drink generous amounts (at least 1 L) of fluid (noncaffeine beverages) to ensure normal body hydration. Hydration status was determined by measuring the specific gravity of urine samples obtained before the test. Room air temperature during all trials averaged  $26 \pm 2.5^{\circ}\text{C}$ .

#### Measurements

Before each trial, body weight was measured, the bladder voided, and venous blood samples taken. After each trial, body weight was again measured, urine volume (UV) collected, and venous blood samples again drawn. The blood samples were analyzed for hematocrit (Hct), hemoglobin (Hb), and plasma proteins. The mean of 4 separate microhematocrit values was used to establish each Hct value used in the calculation of changes in plasma volume percentage ( $\Delta$  PV) which were calculated from pre- and post-trial hematocrit values using the method of van Beaumont.<sup>10</sup> Blood samples were also analyzed for ANP (R&D Antibodies, Berkeley, CA) and ALDO (Diagnostic Products Corporation, Los Angeles, CA) using iodine-125 radioimmunoassay kits. The mean of two samples was used to establish the hormone concentration. All plasma samples were stored at  $-20^{\circ}\text{C}$  before hormone analysis. Oral temperature was measured at hours 0, 1, 2, 3, and 4 of each trial.

Heart rate and respiratory rate were measured throughout each trial using the Medic Monitor ANS-R1000 monitor (Ansar Inc., Philadelphia, PA). In this system, the electrocardiographic (ECG) signal is recorded at 160 Hz, while the respiratory rate is recorded at 4 Hz. The R-to-R intervals from the ECG signal are converted into HR and then converted by linear spline interpolation and re-sampling at 4 Hz into an instantaneous HR. From the generated instantaneous HRs, data segments of 128-sec duration and containing 512 values corresponding to beats per minute (bpm) are constructed by the ANS-R1000 by taking a reading approximately every .25 seconds. After the first segment of 512 bpm values, each successive data segment

comprises 384 values from the previous data segment plus 128 values from the next segment. Spectral analysis was conducted on each 512 value segment to produce real-time high-frequency amplitudes (Rfa) and low-frequency amplitudes (Lfa;  $\text{bpm}^2 \cdot \text{Hz}^{-1}$ ). The amplitude of Lfa represented the spectral power in the frequency range of 0.04 Hz through 0.1 Hz, while the Rfa represented the spectral power in the frequency range encompassing a bandwidth of 0.12 Hz, which is centered at the fundamental respiratory frequency (FRF). Respiratory rate measured in Hz provided the FRF. Additional measures included the Lfa-to-Rfa (LRR) ratio and 32-sec mean-HR (mHR). The LRR ratio has been posited to represent the balance between sympathetic and parasympathetic stimulation.<sup>3</sup>

### Statistical Analysis

A total of 450 data segments (32 sec per segment) of Lfa, Rfa, and mHR values were obtained for each subject over the 4-hr test periods. The data segments were then grouped into 45 temporal phases of 10 segments each. Data for Lfa, Rfa, and mHR associated with a segment FRF that was less than 0.16 Hz (i.e., < 10 breaths per min) or greater than 0.3 Hz (i.e., > 17 breaths per min) were considered anomalous and eliminated from the respective phase. Dependent variable-group differences for the three trials, 45 phases, trial-by-phase interaction were evaluated by repeated-measures analysis of variance (ANOVA). Analyses in the presence of a significant omnibus F ratio included least square means comparisons to evaluate significant differences among trials. The null hypothesis was rejected when  $p < .05$ . All statistical analyses were conducted using Statistical Analysis Systems software (SAS Institute, Inc., Cary, NC).

### **Results**

Eight subjects completed the three trials. Mean and standard deviation values for body weight loss, UV,  $\Delta$  PV, and changes in ALDO and ANP are shown in Table 1. Body weight loss and urine volume were significantly greater for WI when compared with HDT and CON, while differences between HDT and CON were nonsignificant. UV was significantly greater for WI compared to HDT and CON, while differences between HDT and CON were nonsignificant. The percentage changes in plasma volume for HDT and WI over the 4-hr test were significantly greater than that observed among CON. The  $\Delta$  PV between WI and HDT was nonsignificant. Oral temperatures for the three groups ranged from 36.30°C to 36.45°C and were found not to differ significantly. The changes over time in aldosterone concentration for WI and HDT were found to be significantly greater than for CON. There was no significant difference in ALDO between WI and HDT. Also, there were no significant changes in ANP among the trials.

**Table 1.** Mean  $\pm$  SD values for body weight (BW) loss, urine volume output, and changes in plasma volume, aldosterone concentration, and atrial natriuretic peptide for three trial conditions.

<u>Variable</u>	<u>CON</u>	<u>HDT</u>	<u>WI</u>
BW Loss (kg)	-0.519 $\pm$ 0.096	-0.575 $\pm$ 0.085	-0.919 $\pm$ 0.399*
UV Output (ml)	356 $\pm$ 49	401 $\pm$ 142	570 $\pm$ 223*
$\Delta$ PV (%)	-1.86 $\pm$ 5.02	-4.94 $\pm$ 6.77 <sup>†</sup>	-4.31 $\pm$ 6.51 <sup>†</sup>
$\Delta$ ALDO (pg•ml <sup>-1</sup> )	-21.3 $\pm$ 33.0	-83.9 $\pm$ 85.1 <sup>†</sup>	-40.1 $\pm$ 47.4 <sup>†</sup>
$\Delta$ ANP (pg•ml <sup>-1</sup> )	-10.1 $\pm$ 18.5	-4.5 $\pm$ 16.4	-4.8 $\pm$ 12.0

\* indicates significant differences ( $p < .05$ ) when compared to both CON and HDT.

† indicates significant difference ( $p < .05$ ) when compared with CON.

Table 2 presents the effects of trial, time, and trial-by-time interaction in the ANOVAs of Lfa, Rfa, LRR, FRF, and mHR. Means and standard deviation values and significant comparisons among trials are shown in Table 3.

**Table 2.** Trial, Time, and 'Trial-by-Time' effects of Lfa, Rfa, LRR, FRF, and mHR.

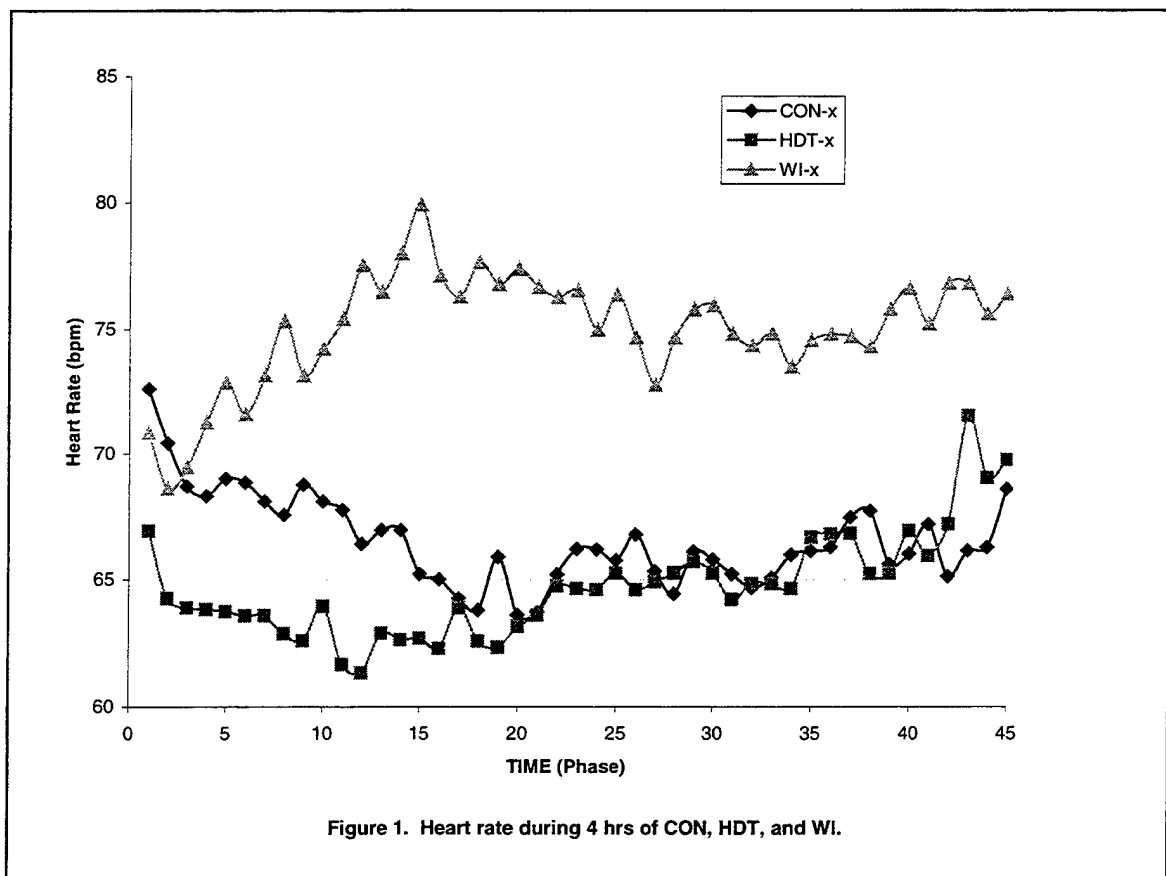
Variable	Trial	Time	Trial x Time
Lfa	$p < 0.05$	n.s.	n.s.
Rfa	$p < 0.05$	n.s.	n.s.
LRR	n.s.	n.s.	n.s.
FRF	n.s.	n.s.	n.s.
mHR	$p < 0.01$	$p < 0.01$	$p < 0.01$



**Table 3.** Mean values of Lfa, Rfa, LRR, FRF, and mHR for the three trial conditions.

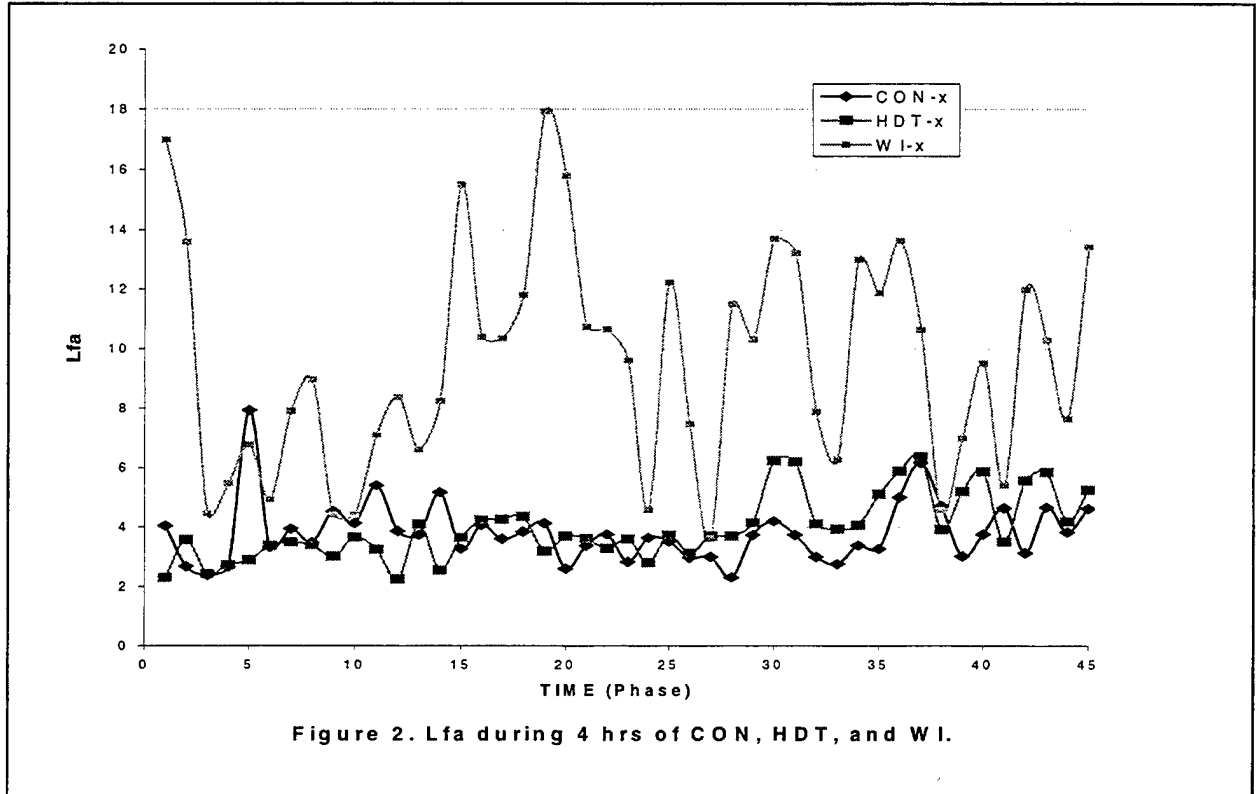
Variable	CON	HDT	WI	Significant ( $p < .05$ )
Lfa	$3.82 \pm 1.04$	$3.78 \pm 0.98$	$11.68 \pm 4.9$	WI > CON, HDT
Rfa	$3.04 \pm 1.35$	$4.46 \pm 1.88$	$11.30 \pm 5.7$	WI > CON, HDT
LRR	$4.39 \pm 0.95$	$4.42 \pm 1.56$	$5.99 \pm 2.66$	n.s.
FRF	$0.24 \pm 0.01$	$0.24 \pm 0.03$	$0.27 \pm 0.01$	n.s.
mHR	$66 \pm 2$	$65 \pm 2$	$75 \pm 2$	WI > CON, HDT

The ANOVA revealed significant trial, time, and trial-by-time effects for mHR with values for WI greater than both CON and HDT. The significant interaction indicates that mHR for WI was gradually increasing throughout the 4-hr test compared with CON and HDT. Before testing began, seated HR averaged  $72 \pm 10$  bpm for all trials. For CON and HDT, mHR decreased to 64 bpm over the first 20 time phases (~106 min). Thereafter, mHR gradually increased to 68 bpm and 70 bpm for CON and HDT, respectively. However, mHR for WI gradually increased during the first 15 phases (~80 min) to 80 bpm, and then stabilized at 74 to 76 bpm for the remainder of the test. Values of mHR for the three trials are shown in Figure 1. Each time phase represents the mean for approximately five minutes of readings (320 seconds).

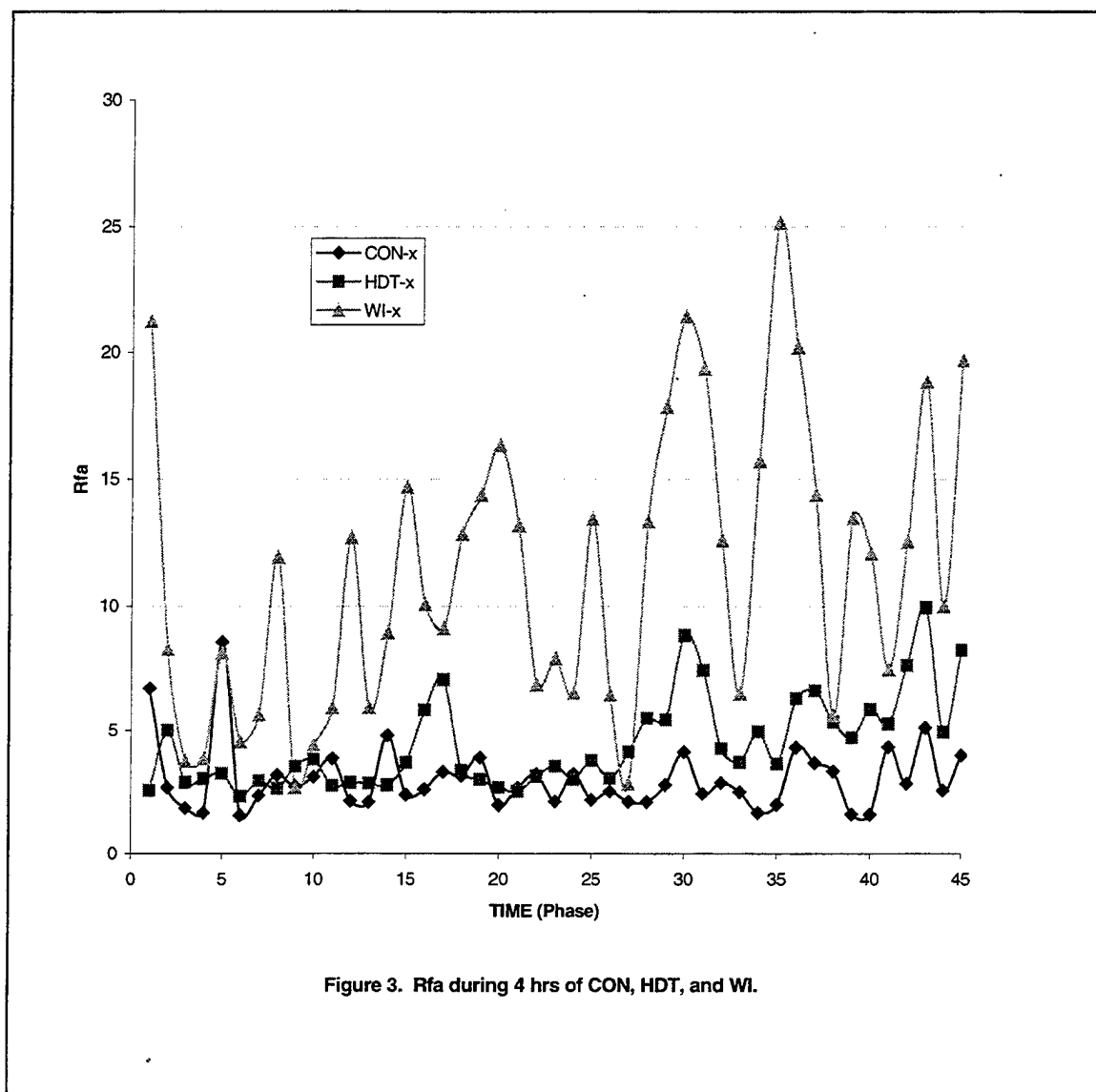


**Figure 1.** Heart rate during 4 hrs of CON, HDT, and WI.

ANOVA revealed significant trial effects for the low-frequency amplitudes, with the overall Lfa for WI significantly higher than for CON and HDT. Lfa for CON and HDT ranged from 2  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  to 6  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  over the 4-hr test, while Lfa for WI fluctuated between 4  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  and 18  $\text{bpm}^2 \cdot \text{Hz}^{-1}$ . Lfa for CON and HDT rose slightly during the latter half of each test, but the time effect was nonsignificant. Lfa of the 45 time phases for the three trials is shown in Figure 2.

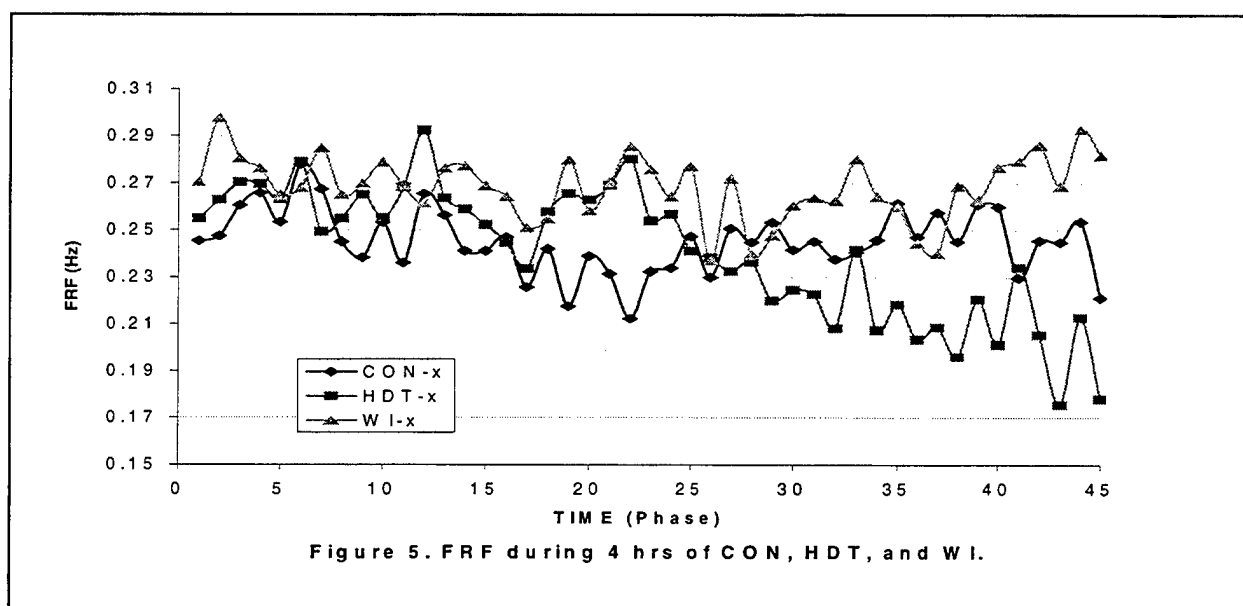
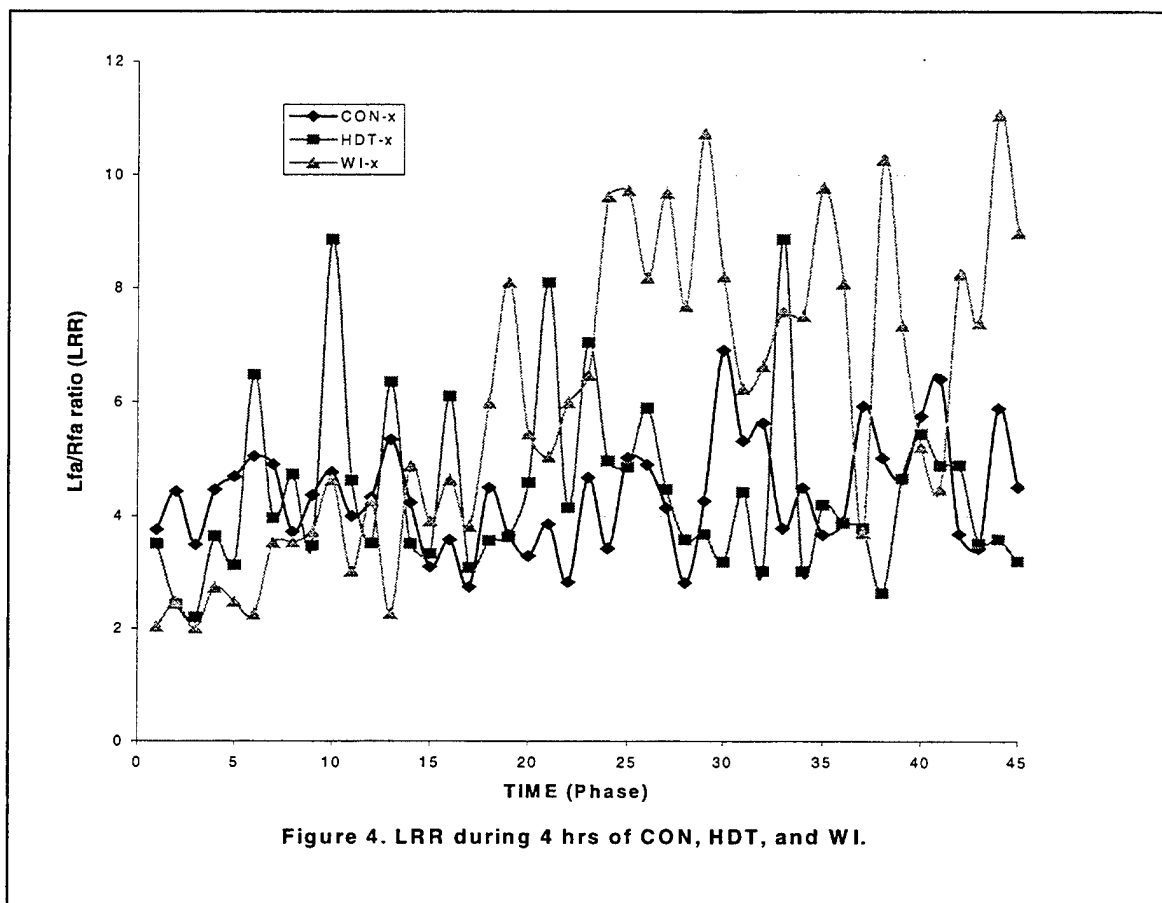


The ANOVA also revealed significant trial effects for the high-frequency amplitudes, with the overall Rfa for WI significantly higher than for CON and HDT. Rfa for CON ranged from 2  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  to 5  $\text{bpm}^2 \cdot \text{Hz}^{-1}$ , and Rfa for HDT ranged from 2  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  to 10  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  over the 4-hr test. Rfa for WI showed a fluctuating response pattern ranging from 3  $\text{bpm}^2 \cdot \text{Hz}^{-1}$  to 25  $\text{bpm}^2 \cdot \text{Hz}^{-1}$ . Rfa for CON and HDT rose slightly during the latter half of each test, but the time effect was nonsignificant. Rfa for the three trial conditions is shown in Figure 3.



The ratio of Lfa to Rfa for CON, HDT, and WI averaged 4.39, 4.42, and 5.99, respectively, over the 4-hr test periods. There was a tendency for the LRR of WI to rise during the last half of the test. However, differences among trials for LRR were nonsignificant. Figure 4 is a display of LRR observed throughout the three trials conditions.

The fundamental respiratory frequency for CON, HDT, and WI averaged  $0.24 \text{ Hz}^{-1}$ ,  $0.24 \text{ Hz}^{-1}$  and  $0.27 \text{ Hz}^{-1}$  respectively, over the 4-hr test periods. There was a tendency for FRF of HDT to decrease during the last half of the test. However, differences among trial conditions for FRF were nonsignificant. Mean FRF for the trial conditions are shown in Figure 5.



## Discussion

Real-time spectral analysis of HRV was used to evaluate the effect of a simulated decrease in blood volume on ANS activity. Previous studies have shown that 'head down tilt' and head-out 'water immersion' in thermoneutral water for extended durations is associated with an increase in central blood volume, diuresis, and a decrease in blood and plasma volume.<sup>6,7,11</sup> Our values for body weight loss, urine volume output, and the percentage decrease in plasma volume indicate that WI and HDT both produced a loss in blood volume and plasma fluid volume.

Four hours of WI produced an average UV of 570 ml, yielding a calculated urine flow rate of  $2.37 \text{ ml} \cdot \text{min}^{-1}$ . Urine flow rates for HDT and the control condition were lower at  $1.67 \text{ ml} \cdot \text{min}^{-1}$  and  $1.48 \text{ ml} \cdot \text{min}^{-1}$ , respectively. The urine flow rates observed for WI were less than the 4.0 to  $6.2 \text{ ml} \cdot \text{min}^{-1}$  flows rates reported by Greenleaf et al.<sup>7</sup> and Deuster et al.<sup>12</sup> for prolonged WI in 35°C water. The urine flow rate for HDT was less than the  $2.7 \text{ ml} \cdot \text{min}^{-1}$  flow rate reported by Shaffer-Bailey et al.,<sup>11</sup> but it was similar to the  $1.66 \text{ ml} \cdot \text{min}^{-1}$  flow rate reported by Hayashi et al.<sup>13</sup> for prolonged HDT.

WI and HDT yielded decreases in PV of 4.31% and 4.94%, respectively, compared with a 1.86% reduction for CON. These values are larger than the 3% to 4% decrease in PV reported by Shi et al.<sup>14</sup> and Williamson et al.<sup>15</sup> for 4 hrs of HDT, but less than the 6% decrease in PV reported by Greenleaf et al.<sup>6</sup> over 4 hrs of WI. WI and HDT in the present study were also associated with decreases in both ALDO and ANP. These responses are similar to those reported by Shi<sup>15</sup> and Williamson et al.,<sup>16</sup> who both reported decreases in ALDO and ANP with for 4 hrs of HDT.

In the present study, body weight losses for CON and HDT were 30% greater than the UV outputs for those conditions, and the body weight loss in the WI condition was 38% greater than the UV output. These differences suggest that additional fluid loss occurred as the result of respiratory water exchange and sweat loss. Our difference for WI is similar to that reported by Greenleaf et al.<sup>7</sup> The higher body weight, UV, and fluid losses for WI are likely the result of elevations in skin temperature (that were clamped at the 35°C water temperature), and subsequent demand for a high skin blood flow.<sup>16</sup>

The WI protocol produced a significant increase in the mean heart rate that remained elevated throughout the 4 hrs of the test. This response pattern is opposed to that found by Miwa et al.<sup>8</sup>

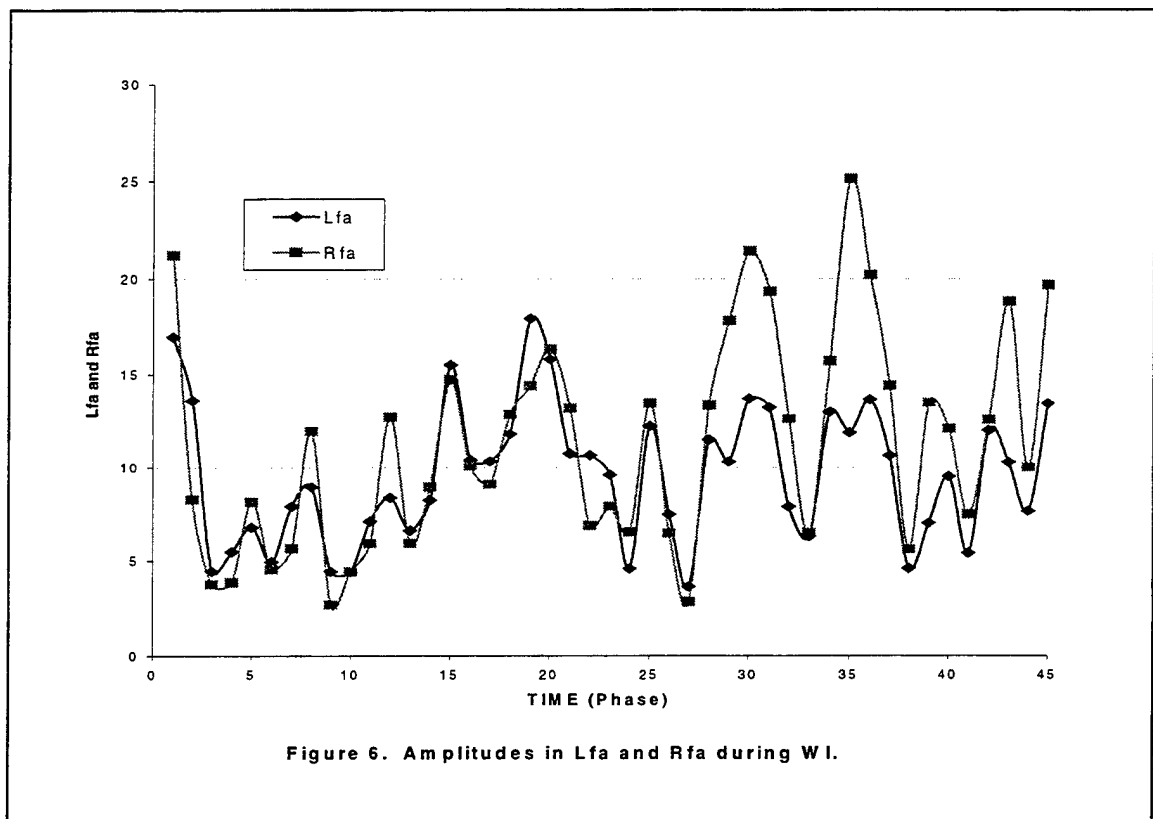
who reported that 5 min of WI decreased HR and increased stroke volume and cardiac output. The mHR response for WI in the present study also is in contrast to that observed for HDT and CON, which both decreased to 62 - 64 bpm, and then stabilized at values ranging from 65 bpm to 70 bpm. The decrease in mHR during HDT is similar to that reported by Butler et al.<sup>17</sup> who reported that HDT also increased stroke volume and cardiac output.

During WI, skin temperature was clamped at 35°C via maintenance of a constant water temperature in the swim flume in which the subjects were seated. Controlling skin temperature at this level was done to prevent chilling over the 4 hrs of water immersion and to make the subject comfortable. However, a skin temperature of 35°C in air of 21°C would tend to increase the heat exchange between the subject and the air. Greenleaf et al.<sup>6,16</sup> reported increases in respiratory water loss and sweat rates, and higher skin blood flows in subjects immersed in such conditions. Thus, the higher mHR for WI is likely related to an increase in skin blood flow to prevent a gain in body heat content and maintain thermal balance. An elevation in mHR during WI in response to an increased demand of heat stress is supported by the increase in respiratory water and sweat loss.

The elevated mHR for WI was accompanied by an increase in Lfa, which fluctuated between 4 bpm<sup>2</sup>•Hz<sup>-1</sup> and 18 bpm<sup>2</sup>•Hz<sup>-1</sup>, and an increase in Rfa, that fluctuated from 3 bpm<sup>2</sup>•Hz<sup>-1</sup> and 25 bpm<sup>2</sup>•Hz<sup>-1</sup>. Interestingly, the values for Rfa-to-Lfa ratio during WI did not reveal the magnitude of the Lfa and Rfa responses or their fluctuating response patterns. The response pattern for WI is opposite from that found by Miwa et al.<sup>8</sup> who reported that the decrease in HR with WI was accompanied by an increase in HF (i.e., Rfa) and a decrease in LF (i.e., Lfa) spectral amplitudes. However, our results support those of Perini et al.<sup>9</sup> who reported that 5 min of WI was associated with large increases in both HF (i.e., Rfa) and LF (i.e., Lfa). The major limitation of these earlier studies, however, is the short duration of WI, and the absence of demonstrated changes in body fluid volumes. The Lfa and Rfa responses for WI were in contrast to those of HDT and CON, which were generally lower in value and devoid of large fluctuations in response.

The large amplitudes for Lfa and Rfa during WI suggest that control of HR occurred through both sympathetic and respiratory-mediated baroreflex-modulated vagal activity. In addition, as seen in Figure 6, the response patterns of Lfa and Rfa during WI indicate that the fluctuations in these two measures were largely contemporaneous. The temporal contiguity of the Lfa and Rfa amplitudes suggests that the increase in mHR may be the result of sympathetic stimulation in conjunction with a near-simultaneous baroreflex modulation of parasympathetic activity. During immersion,

hydrostatic pressure on the legs and trunk are increased, forcing blood from the peripheral veins into the thorax increasing central (pulmonary) blood volume and capacities.<sup>18</sup> Water immersion also causes compression of the abdomen and an upward movement of the diaphragm.<sup>19</sup> These adjustments could modify respiratory patterns and enhance modulation of baroreflex-mediated vagal activity, thereby yielding an increase in HRV.<sup>9</sup>



In the present study, mean LRR did not differ significantly among the CON, HDT, and WI groups. In addition, LRR did not reveal the magnitude of the Lfa and Rfa responses or the pattern of the responses among the trial conditions, and thus, was not useful in identifying sympathetic and parasympathetic responses. The similarities of LRR response among the three tests suggest that LRR may lack sensitivity as an indicator of changes in sympathetic and parasympathetic activity. While, LRR has been proposed as an index of ANS balance,<sup>3</sup> the results of the present study did not bear this out.

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<b>1. Report Date (DD MM YY)</b> 09/01/00	<b>2. Report Type</b> Final	<b>3. DATES COVERED (from - to)</b> 10/01/99 to 9/30/00
<b>4. TITLE AND SUBTITLE</b> Heart Rate Variability and Changes in Blood Volume		<b>5a. Contract Number:</b> <b>5b. Grant Number:</b> <b>5c. Program Element:</b> 64771N <b>5d. Project Number:</b> M2443 <b>5e. Task Number:</b> <b>5f. Work Unit Number:</b> 6901
<b>6. AUTHORS</b> RD Hagan, MJ Buono, S Singh, CG Blood		<b>9. PERFORMING ORGANIZATION REPORT NUMBER</b> Report No. 00-40
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Naval Health Research Center P.O. Box 85122 San Diego, CA 92186-5122		
<b>8. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)</b> Office of Naval Research      Chief, Bureau of Medicine and Surgery 800 North Quincy Street      Code: BUMED-26 Arlington, VA 22217-5600      2600 E St NW Washington DC 20372-5300		<b>10. Sponsor/Monitor's Acronyms(s)</b> ONR/BUMED
		<b>11. Sponsor/Monitor's Report Number(s)</b>

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**13. SUPPLEMENTARY NOTES****14. ABSTRACT (maximum 200 words)**

Real-time spectral analysis of heart rate variability (HRV) was used to evaluate the effect of a simulated decrease in blood volume on ANS activity. Previous studies have shown that 'head down tilt' (HDT) and head-out 'water immersion' (WI) in thermoneutral water for extended durations is associated with an increase in central blood volume, diuresis, and a decrease in blood and plasma volume. Our values for body weight (BW) loss, urine volume (UV) output, and the percentage decrease in plasma volume (PV) indicate that WI and HDT both produced a loss in blood and plasma fluid volume. In the present study, mean LRR (ratio of low frequency component to high frequency component) did not differ significantly among the CON (control), HDT, and WI groups. In addition, LRR did not reveal the magnitude of the Lfa (low frequency component) and Rfa (high frequency component) responses or the pattern of the responses among the trial conditions, and thus, did not prove useful in identifying sympathetic and parasympathetic responses. The similarities of LRR response among the three tests suggest that LRR may lack sensitivity as an indicator of changes in sympathetic and parasympathetic activity. While LRR has previously been proposed as an index of ANS balance, the results of the present study provide no additional support to this notion.

**15. SUBJECT TERMS**

<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b> UNCL	<b>18. NUMBER OF PAGES</b>  17	<b>19a. NAME OF RESPONSIBLE PERSON</b> Commanding Officer
<b>a. REPORT</b> UNCL	<b>b. ABSTRACT</b> UNCL	<b>b. THIS PAGE</b> UNCL			<b>19b. TELEPHONE NUMBER (INCLUDING AREA CODE)</b> COMM/DSN: (619) 553-8429